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Post-mortem Lability of Skeletal Muscle Proteins

As a result of the post-mortem conversion of glycogen to lactic acid, the *p*H of skeletal muscle falls from its *in vivo* value of about 7·3 to about 5·5, the actual level attained depending on several factors, such as the physiological state of the animal before death, the type of muscle and the temperature during glycolysis[1,2]. It has recently been shown in pig muscle that, where the rate of *p*H fall is fast and a relatively low *p*H is therefore attained while the temperature is still high, there may be considerable denaturation and precipitation of sarcoplasmic proteins[3]. It is also well known in enzyme preparatory procedures that lowering of the *p*H of extracts from animal tissues precipitates particulate material and nucleoprotein[4].

Giles[5], Tsuyuki[6] and others have used vertical starch-gel electrophoresis to separate sarcoplasmic proteins of various species. These were extracted from muscles which in some cases had already attained a low *p*H, however; it is thus likely that certain components had been denatured, were not extracted and did not appear on subjecting the extracts to starch-gel electrophoresis. The present investigations, in which a similar technique was used, have clearly indicated that certain sarcoplasmic proteins are unstable *post mortem*; precipitation of even small amounts of these can inhibit the normal extraction behaviour of muscle protein fractions[3,7].

Beef l. dorsi muscle was obtained immediately after slaughter and had been chilled to 0° C. within 1 h of death. Samples were held at 0° C for 20 h, at 37° C for 4 h, or used at once. Homogenates were extracted with distilled water, after adjusting the *p*H to 7·0 with M *tris*, and the extracts were subjected to starch-gel electrophoresis. Some 35 bands can be obtained from beef muscle; these are diagrammatically represented in Fig. 1. In Fig. 2, it can be seen that several components are removed completely or very much diminished by the fast post-mortem glycolysis which occurs at 37° C in comparison either with the initial condition (before post-mortem glycolysis had proceeded appreciably) or with muscle in which post-mortem *p*H fall has been relatively slow (that is, at 0° C). Even in extracts prepared from muscles which have had a slow rate of *p*H fall, there are minor differences compared with those from the initial material of high *p*H, and it is evident

that at the normal ultimate *p*H of muscle—5·5—certain sarcoplasmic proteins are already denatured and immobile.

Fig. 3 demonstrates that the major component of pig muscle sarcoplasmic proteins which migrates towards the anode at *p*H 8·5. and corresponds to a similar major

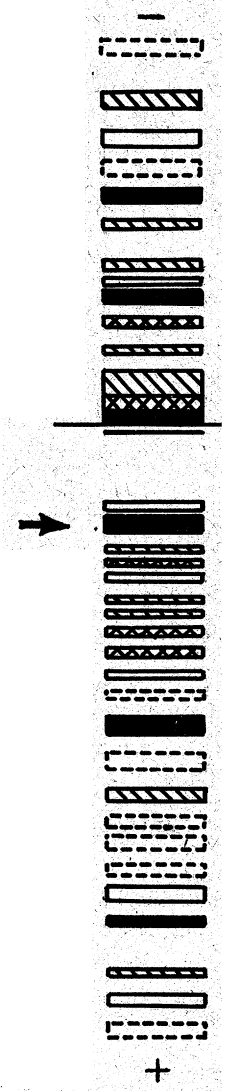

Fig. 1. Composite pattern of bands from beef muscle sarcoplasmic protein after starch-gel electrophoresis

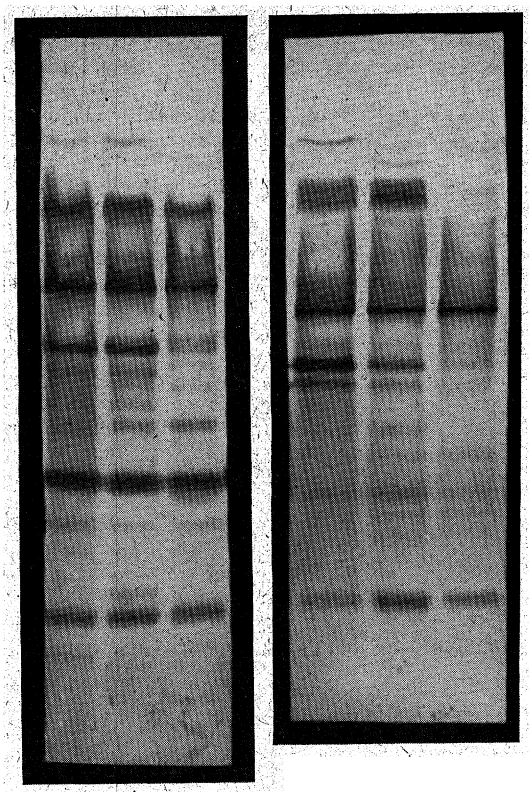

Fig. 2 Fig. 3

Fig. 2. Electrophoretogram of beef muscle sarcoplasmic proteins. Left to right: extract of pre-rigor muscle; extract of muscle which was held at 0° for 20 h *post mortem*; extract of muscle which was held at 37° for 4 h *post mortem*

Fig. 3. Electrophoretogram of pig muscle sarcoplasmic protein. Left to right: (*a*) normal pig muscle; (*b*) 'white muscle disease' pig muscle; (*c*) severe 'white muscle disease' pig muscle

component in electrophoretograms of beef muscle extract (arrowed in Fig. 1), and to band 9 in those of rabbit muscle (as tabulated by Hartshorne and Perry[8]), is markedly affected by the high-temperature/low *p*H combination. All these samples were prepared from post-rigor pig l. dorsi muscle; samples (*b*) and (*c*), however, were obtained from pigs having 'white muscle disease', in which the *p*H dropped rapidly *post mortem*, while the temperature was still high[3]. This caused considerable denaturation and insolubility of this sarcoplasmic com-

right; some other proteins affected are also clearly demonstrated. Sample (*b*) shows some variation from the other two which may be attributable to breed differences.

Precipitation of the '*p*H 5' proteins[9], by lowering the *p*H of sarcoplasmic extracts, produces a fraction which, on re-solution at *p*H 7·5, corresponds for the most part with the components most affected by the high-temperature treatment. It is concluded that *in situ* isoelectric precipitation of these proteins renders them more susceptible to heat denaturation. Most of them are still extractable on raising the *p*H to 7·0 providing that the temperature had not been high during the post-mortem *p*H fall.

Thus, it has been shown that several protein components of the sarcoplasmic complex are unstable and either denature, or are isoelectrically precipitated by the post-mortem *p*H fall in muscle, both if this is extensive (low ultimate *p*H) and/or fast. Many of the protein constituents are completely stable, however, and show no diminution in the starch-gel patterns, under these conditions.

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Note added in proof. The major component referred to (arrowed in Fig. 1) has been identified as creatine phosphoryltransferase in both pig and beef muscle. It is not, however, found in the '*p*H 5' proteins.

R. K. Scopes
R. A. Lawrie

Low Temperature Research Station,
Cambridge

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